Identifying germination cues for seven Basalt Plains grassland species prior to their use in a field sowing

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Abstract

Germination characteristics of seven indigenous grassland species from the Victorian (Western) Basalt Plains (*Austrodanthonia racemosa, Bulbine bulbosa, Chrysocephalum apiculatum, Leptorhynchos squamatus, Leucochrysum albicans* ssp. *albicans* var. *tricolor, Linum marginale, Wahlenbergia stricta*) were investigated prior to their use in a seeding program. Germination tests at 20°/10°C and 12hr/12 hr light/dark showed a range of maximum germination responses from 21% (*W. stricta*) to 83% (*A. racemosa*). Two trends were observed in terms of speed of germination. *Austrodanthonia racemosa, Chrysocephalum apiculatum* and *Linum marginale* germinated rapidly (t_{50} < 7 and 14 days) and *Bulbine bulbosa, Leptorhynchos squamatus, Leucochrysum albicans* ssp. *albicans* var. *tricolor* and *Wahlenbergia stricta* exhibited a slower germination response (t_{50} between 20 and 30 days). When seeds of each species were exposed to smoke product, cool stratification and smoke cool + stratification, *Linum marginale* was the only species to respond with significantly higher germination (smoke application). Further testing of smoke product at recommended and higher rates produced a range of species-specific dose responses, both positive and negative. These findings confirmed considerable species-specific variations in responses, and differences/similarities of responses between ours and other studies. Therefore, to most accurately gauge seed-lot characteristics, it is recommended that species are routinely tested prior to their use in grassland restoration programs. (*The Victorian Naturalist* **127** (5) 2010, 184-191).

Keywords: grassland restoration, seed germination, seed testing, smoke, direct-seeding

Introduction

The Basalt Plains grasslands of south-western Victoria are a sub-set of the larger Australian south-eastern lowland temperate grasslands. They currently exist as small and fragmented remnants distributed over an area of approximately 23 000 km^2 of flat and undulating lands (approximately 1% of their original range). These grassy communities are listed as threatened under the *Victorian Flora and Fauna Guarantee Act* (1988) and the *Commonwealth Environment Protection and Biodiversity Conservation Act* (1999). They also have been recognised by the Federal Government's Threatened Species Scientific Committee as one of 15 national biodiversity 'hotspots'.

Basalt Plains grasslands are a diverse flora characterised by tussock grasses, primarily Kangaroo grass *Themeda triandra*, Wallaby grass *Austrodanthonia* sp. and Spear grass *Austrostipa* sp., with a wide variety of native forbs and both annual and perennial exotic species (grasses and forbs) occupying the inter-tussock spaces. A variety of other organisms from different trophic levels contribute to their function and stability, including mosses, lichens, liverworts and algae (that form cryptogamic crusts on soil surfaces), soil-inhabiting arbuscular mycorrhizal fungi, invertebrate species and a host of vertebrates, reptiles, birds and mammals.

Initial attempts to restore or reconstruct these grasslands were primarily undertaken through the re-introduction of container grown plant material (Hitchmough 1994; Morgan 1999). However, direct seeding has increasingly become the focus of investigation and implementation as a restoration technique (Morgan *et al.* 1993; Huxtable and Whalley 1999; Gibson-Roy 2005; Gibson-Roy *et al.* 2007b). There are constraints with both methods on the availability and knowledge relating to the quality of seed from a broad range of grassland species (Huxtable and Whalley 1999; Mortlock 2000; Gibson-Roy and Delpratt 2006).

Where available, seed resources must be used efficiently (whether for propagation or for direct seeding). To this end, restorationists require information about seed-lot quality and

the germination characteristics of the species to be used (Gardiner and Midgley 1994; Delpratt 1996). Earlier formal studies examining simulated environmental cues have revealed a range of responses by species from this flora to diurnal light, stratification, smoke, fluctuating temperatures, storage environments and storage periods (Willis and Groves 1991; Morgan and Lunt 1994; Dixon *et al.* 1995; Morgan 1998; Bell 1999; Clarke *et al.* 2000; Gibson-Roy *et al.* 2007a). In practice, grassland seed is seldom tested for quality prior to its use in restoration programs, potentially limiting the success and/or interpretation of outcomes (Gibson-Roy and Delpratt 2006).

The aim of this study was to test the response of seven grassland species to a range of simulated germination cues for an indication of possible field responses. Seed testing sought to establish the germination response of each species when exposed to:

- projected autumn temperatures;
- cool-stratification and autumn temperatures;
- plant derived smoke (at various rates).

Methods

Seven indigenous grassland species (*Austrodanthonia racemosa, Bulbine bulbosa, Leptorhynchos squamatus, Chrysocephalum apiculatum, Leucochrysum albicans* ssp. *albicans* var. *tricolor, Linum marginale, Wahlenbergia stricta*) representing a range of functional groups from the Victorian (Western) Basalt Plains grassland community (C3 Grass, Geophyte, Hemicryptophyte, Chamaephyte) were selected for a seeding project. Seed was sourced from roadside populations occurring on basaltic soils near Cressy in Victoria's South West in spring and summer. Seed from two species was sourced from production crops grown at Burnley College. After harvest, seed-lots were dried and stored in paper bags at room temperature until testing (and subsequent sowing in the following autumn).

Germination test

All seed lots were cleaned by removing unattached inert matter. Based on the appearance of individual propagules, seed-lots were then sampled for filled and unfilled seeds. To determine if plumpness was a reliable indicator of the presence of an embryo, sub-samples of 100 seeds from each species were randomly chosen for inspection and dissection under a binocular microscope (Olympus SZ x 20). Dissection confirmed that visual assessment was a reliable predictor of filled seed.

For testing, six replicates of 25 filled seeds (of each species) were sampled. Seeds were placed on white absorbent paper laid over towelling moistened with deionised water within lidded rectangular plastic trays (210 mm x 310 mm x 30 mm). One tray per species was placed into a germination cabinet set to alternating day/night temperatures of 20°C/10°C, with 12 hr light (supplied by four high voltage fluorescent tubes and four incandescent lights) and 12 hr dark during each 24 hr period. Each tray was inspected at four-day intervals for 43 days. All trays were rotated after each count to spread the effect of any variations in light intensity and temperature within the cabinet. No fungicides were applied to the seeds during the germination test.

Germination was recorded as the emergence of a radicle. Germinated seeds were removed after each inspection. For each species, the following germination characteristics were determined:

- percentage germination after 20 days (G_{20}) and 43 days (G_{43});
- percentage increase in germination from day 20 to day 43;
- time elapsed (lag) between the start of the test (imbibition) and the first germination. Species were grouped into the following lag responses: <7 days, 7-14 days, 15-28 days, >28 days;
- time elapsed between imbibition and 50% of final germination (t_{50}). Species were grouped into the following t_{50} responses: <7 days, 7-14 days, 15-28 days, >28 days.

Glasshouse Experiment 1. Effects of cool stratification and smoke on seedling emergence

A glasshouse experiment investigated the effect of cool stratification and the application of commercial smoke vermiculite (Grayson Regen 2000® Seed Germinator) on the emergence of seedlings of each species. The experimental units were 340 mm x 320 mm black plastic seedling trays. In each tray, the seeds of all species were sown in rows at 40 mm intervals onto a pine bark propagation medium. Sown seeds were covered with either medium grade vermiculite or a blend of medium grade vermiculite and Grayson Regen 2000® Seed Germinator smoke vermiculite (of similar particle

size). The position of species within each tray was randomly allocated. The sowing density for each species was dictated by propagule morphology and size. If seeds were easily cleaned and counted to individuals, 25 seeds were sown in each row. If protective or dispersal coverings made it difficult to isolate individual seeds, a known mass was sown in each row to achieve a target sowing of approximately 25 propagules. Treatments were applied to trays randomly and each treatment was replicated four times. Trays were placed on a glasshouse bench in four randomised blocks. All trays were watered twice daily during the study period. The number of seedlings was recorded weekly for 89 days. Seedlings were not removed after emergence.

The following experimental treatments were imposed.

1. Control:- sown tray lightly covered with 200 mL of vermiculite.
2. Cool stratification:- sown tray lightly covered with 200 mL of vermiculite, watered and placed into a cool room at approximately 4°C for 14 days prior to transfer to the glasshouse.
3. Smoke:- sown tray covered with a blend of 140 mL of vermiculite and 60 mL of Grayson Regen 2000® Seed Germinator smoke vermiculite.
4. Cool stratification + smoke:- sown seed prepared as per the smoke treatment; trays were placed into a cool room at approximately 4°C for 14 days prior to transfer to the glasshouse.

Glasshouse Experiment 2. Effects of differing formulations and rates of application of smoke vermiculite

This experiment investigated the effect of differing application rates of the commercial smoke products Grayson Regen 2000® Seed Germinator smoke vermiculite and Grayson Regen 2000® Seedstarter smoke vermiculite on the emergence of seedlings of each species.

The seed for this experiment was prepared in the manner described for the Glasshouse Experiment 1. Each experimental unit was a 100 x 100 mm black plastic propagation tray. Seeds were sown onto a pine bark propagation medium at the rates described in Glasshouse Experiment 1. Experimental units were randomly allocated within three replicate blocks on a bench in an unheated glasshouse. All containers were watered automatically twice daily. The number of seedlings was recorded weekly for 89 days. Seedlings were not removed after emergence.

The following experimental treatments were imposed.

1. Control:– no smoke product.
2. Smoke concentration 1:– Grayson Regen 2000® Germinator @ 600 mL m^{-2} (manufacturer's recommended application rate).
3. Smoke concentration 2:– Grayson Regen 2000® Seedstarter @ 18 mL m^{-2} (equivalent to application rate in Treatment 2).
4. Smoke concentration 3:– Grayson Regen 2000® Seedstarter at 180 mL m^{-2} (ten times the smoke product concentration of Treatments 1 and 2).

The data for each study were tested for normality using Ryan-Joiner and, where required, log transformed to satisfy heterogeneity of variance. Differences were compared using ANOVAs with the statistical software Minitab 15.1.

Results

Germination test

Germination occurred for all seven species. However, there were differences between species in the speed of germination and in the final germination percentage (Fig. 1). At day 43 *Austrodanthonia racemosa* and *Chrysocephalum apiculatum* exhibited the highest germination figures (>80%), while *Wahlenbergia stricta* recorded the lowest germination (21%). *A. racemosa, C. apiculatum* and *Linum marginale* germinated quickly, reaching between 40% and 60% germination within 5 to 10 days of imbibition. Conversely, although *Bulbine bulbosa, Leptorhynchos squamatus* and *Leucochrysum albicans* began to germinate within 10 days of imbibition, each reached less than 10% by day 15. *Wahlenbergia stricta* germinated from day 16 but total germination reached only 21% by day 43. Four species, *B. bulbosa, Leptorhynchos squamatus, Leucochrysum albicans* and *W. stricta* showed significant (>100%) increases in germination between days 20 and 43. However, of these species only *Leucochrysum albicans* achieved final germination greater than 50% (Table 1).

Glasshouse experiment 1. Effects of stratification and smoke

All species germinated under the experimental treatments. Only *Linum marginale* exhibited significantly increased emergence in response to any treatment. For that species, the stratifica-

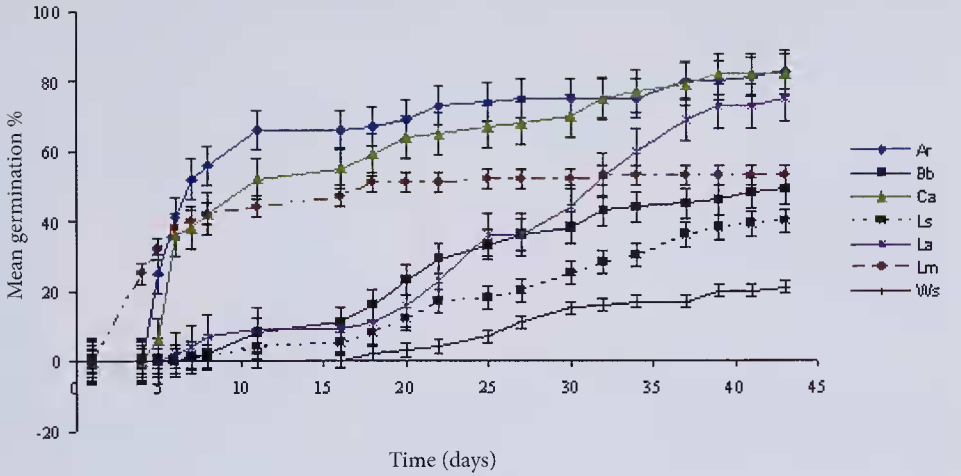

Fig. 1. Mean germination (+/- SE) of seven grassland species over 43 days under cabinet conditions of alternating temperature (20°/10°C) and light (12 hr/light 12 hr/dark). Ar = *Austrodanthonia racemosa*; Bb = *Bulbine bulbosa*; Ca = *Chrysocephalum apiculatum*; Ls = *Leptorhyncos squamatus*; La = *Leucochrysum albicans* spp. *albicans* var. *tricolor*; Lm = *Linum marginale*; Ws = *Wahlenbergia stricta*.

tion (11[b]), smoke + stratification (15[b]) and the smoke treatment (23[c]) gave enhanced emergence ($p < 0.05$) over the control treatment (5[a]). However, the smoke and the stratification + smoke treatments resulted in the highest germination.

Glasshouse Experiment 2. Effects of differing formulations and rates of application of smoke vermiculite

Except for *Wahlenbergia stricta*, seedlings of all species emerged during this experiment (Table 2). For emerging species, results were similar in treatments common to this experiment and Glasshouse Experiment 1. There was also close agreement between the results for treatments that compared the two formulations of smoke vermiculite when they were applied at the rate recommended by the manufacturer. As was the case in Glasshouse Experiment 1, only *Linum marginale* responded positively to the smoke products at recommended rate (Regen 2000® Germinator - 600 mL m^{-2} and Regen 2000® Seedstarter - 18 mL m^{-2}).

The application of smoke product at 10 times the recommended rate (Grayson Regen 2000® Seedstarter - 180 mL m^{-2}) significantly reduced the number of seedlings of *Austrodanthonia racemosa, Chrysocephalum apiculatum, Leucochrysum albicans* and *Linum marginale* ($p < 0.05$). There was no significant effect on seedling numbers of *Leptorhynchos squamatus*. The number of seedlings of *Bulbine bulbosa* increased significantly in trays treated with this higher rate of Grayson Regen 2000® Seedstarter ($p < 0.05$).

Discussion

The restoration of threatened grassland communities will require access to, and knowledge of, a scarce seed resource. The seven species examined in this study are representatives from this florally diverse community and each is likely to be included in restoration programs. The tests conducted in this study revealed a range of species-specific responses to temperature and smoke cues that may guide plant propagators and restorationists undertaking propagation and seeding programs.

Germination cabinet testing

All seven species were germinable under a simulated autumn/spring 20°/10°C temperature regime. Species-specific responses were highlighted through differences in speed of germination and the final germination percentage. These observations are consistent with the findings of McIntyre (1990), Morgan and Lunt (1994), Morgan (1998), and Gibson-Roy *et*

Table 1. Germination responses of seven grassland species at day 43 under alternating temperature (20°/10°C) and light (12 hr light/12 hr dark). G_{20} = mean % germination after 20 days; G_{43} = mean % germination after 43 days; $G_{20\text{-}43}$ = % increase in germination from day 20 to day 43; LAG (days) = days elapsed between imbibition and first germination; t_{50} (days) = days elapsed between imbibition and 50% of final germination.

Genus/Species	G_{20} (%)	G_{43} (%)	$G_{20\text{-}43}$ (%)	LAG (days)	t_{50} (days)
Austrodanthonia racemosa	69	83	20	<7	<7
Bulbine bulbosa	23	49	113	7-14	15-28
Chrysocephalum apiculatum	64	82	28	<7	7-14
Leptorhynchos squamatus	12	40	233	7-14	15-28
Leucochrysum albicans	16	75	369	<7	15-28
Linum marginale	51	53	4	<7	<7
Wahlenbergia stricta	3	21	600	7-14	15-28

Table 2. Mean seedling numbers (Standard Deviation) at the completion of the Glasshouse Study 2 imposing differing rates of smoke vermiculite. Control = no application of smoke vermiculite; smoke 1 = Grayson Regen 2000® Germinator @ 600 mL m^{-2}; smoke 2 = Grayson Regen 2000® Seedstarter @ 18 mL m^{-2}; smoke 2 (high) = Grayson Regen 2000® Seedstrater @ 180 mL m^{-2}. Different letters indicate significant differences within rows following One-Way ANOVA ($p < 0.05$). ns = not significant.

Genus/Species	Control	Smoke 1	Smoke 2	Smoke 2 (high)
Austrodanthonia racemosa	37^{b} (1.5)	38^{b} (4.6)	36^{b} (1.8)	10^{a} (3.0)
Bulbine bulbosa	5^{a} (1.5)	10^{b} (0.3)	7^{a} (2.5)	15^{b} (1.5)
Chrysocephalum apiculatum	40^{b} (13.5)	62^{b} (14.6)	54^{b} (7.1)	21^{a} (7.5)
Leptorhynchos squamatus	20^{ns} (9.0)	18^{ns} (3.5)	14^{ns} (1.0)	11^{ns} (2.5)
Leucochrysum albicans	23^{c} (0.8)	18^{bc} (0.5)	16^{b} (2.5)	11^{a} (2.5)
Linum marginale	8^{b} (0.2)	18^{c} (0.7)	16^{c} (1.1)	1^{a} (0.5)
Wahlenbergia stricta	0	0	0	0

al. (2007a). It is unlikely that any single set of temperature and/or light regimes will provide optimal germination conditions for a broad selection of grassland species. This is an important issue for field sowings of complex species mixes. It is unrealistic to expect rapid and synchronous emergence of all species under any combination of field conditions.

In general, the species exhibited two patterns in terms of the speed at which germination occurred. One group (*Austrodanthonia racemosa, Chrysocephalum apiculatum, Leucochrysum albicans, Linum marginale*) began to germinate rapidly (lag < 7 days). The second group (*Bulbine bulbosa, Leptorhynchos squamatus* and *Wahlenbergia stricta*) were slower to germinate (lag 7-14 days). These responses are consistent with results reported by Morgan (1998), who found that *C. apiculatum* and *Leucochrysum albicans* had lag times of < 7 days, *Leptorhynchos squamatus* a lag of 7-14 days and that *B. bulbosa* exhibited a lag of 14-28 days.

Interestingly, a short lag period did not always result in a high final germination percentage. For example, *Linum marginale* germinated rapidly until day seven (40%), after which little further germination occurred (53% at day 43). This is a commonly observed pattern in recently-harvested seed of this species (John Delpratt unpub. data). Conversely, there were few germinants of *Leucochrysum albicans* up to day 15 (10%), after which germination increased rapidly, reaching 75% by day 43. However, we are aware that the lag (and t_{50}) period for *Leucochrysum albicans* reduces to a few days following longer periods of dry storage (> 3 months) (Gibson-Roy and Delpratt, unpub. data).

Comparison of our results with those of similar studies indicates that testing of the same species can result in both similarities and differences in germination responses. For example, final percentage germination for *Austrodanthonia racemosa* was (G_{43}) 83%. This is similar to Gibson-Roy *et al.* (2007a) (G_{28} 95%). *C. apiculatum* (G_{43} 82%) was similar to Morgan (1998) (G_{56} 87%), and Willis and Groves (1991) (G_{60} 94%). Conversely, *Leptorhynchos squamatus* (G_{43} 40%) exhibited a higher germination percentage than reported by Morgan (1998) (G_{56} 20%) and Gibson-Roy *et al.* (2007a) (G_{28}

16%), as did *W. stricta* (G_{43} 21%) compared to Willis and Groves (1991) report of 0% germination after 60 days. Our finding of 49% (G_{43}) germination for *B. bulbosa* was lower than Morgan (1998) (G_{56} 74%), but higher than that reported by Gibson-Roy *et al.* (2007a) (G_{28} 14%). These differences in germination responses between tests and studies can result from differences in maternal conditions, seed collection techniques, cleaning and storage techniques, cabinet environments and observation periods. This confirms that the testing of seed-lots prior to their use in restoration programs will give a more accurate indication of germinability than will historical information for that species.

Glasshouse experiments

Cool stratification and smoke

Dixon *et al.* (1995) reported that the germination of many Western Australian species could be promoted by the application of plant-derived smoke. Since then, a number of other studies have reported varying responses to smoke cues across a broad range of Australian forest and understorey species (Roche *et al.* 1997; Tieu *et al.* 1999; Clarke *et al.* 2000; Lloyd *et al.* 2000; Merritt *et al.* 2006; Thomas *et al.* 2007). Such studies indicate that while a number of species show a positive response to smoke derivatives, many others exhibit no, or only a small, response to smoke or smoke derivatives under laboratory or glasshouse conditions (Clarke *et al.* 2000; Merritt *et al.* 2007).

In Glasshouse Experiment 1, the emergence of *Linum marginale* was significantly enhanced above that of the control treatment (20%), using smoke vermiculite, cool stratification and a combination of smoke vermiculite and cool stratification. However, the smoke treatment on its own resulted in significantly higher emergence (92%) than smoke + stratification (60%) and the stratification (44%). This information, coupled with the lack of evidence for any serious inhibition of the other species, suggests that the application of smoke might be considered when propagating this species.

For the other species, neither smoke application, cool stratification, nor a combination of both, resulted in enhanced seedling emergence above that of a control treatment. Again, these responses are not always consistent with findings from earlier testing. For example, Clarke *et al.* (2000) reported lower emergence of *Bulbine bulbosa* and *Leucochrysum albicans* after seed had been exposed to cool stratification. In the case of *L. albicans*, Willis and Groves (1991) found it had the effect of increasing germination. Our findings suggest that cool stratification prior to propagation or seeding would not enhance germination or emergence of these six grassland species.

In Glasshouse Experiment 2, we tested the effects of smoke products at recommended and higher rates. As was expected, we observed a significant enhancement of germination over the control treatment for *L. marginale* using recommended rates of Grayson Regen 2000® Germinator (600 mL m^{-2}) and Grayson Regen 2000® Seedstarter (18 mL m^{-2}) treatments. This confirmed the finding of Glasshouse Experiment 1 and further indicates that the germination of this species can be stimulated by the application of either of these commercial formulations (at recommended rates). Interestingly, *Wahlenbergia stricta* failed to emerge under any treatment. The reason is not clear as seeds from the same seed-lot germinated/emerged (albeit at low rates) in the other two experiments and in subsequent field experiments (Gibson-Roy 2000). It is possible that the dormancy status of seed from this species had increased by the time of this experiment, suggesting that this species may experience dormancy cycling (Baskin and Baskin 1998).

Consistent with Glasshouse Experiment 1, other than *Linum marginale*, our species did not respond significantly to smoke at the recommended application rates (both Grayson Regen 2000® Germinator and Grayson Regen 2000® Seedstarter). However, when compared to the control treatment, emergence of *Leucochrysum albicans* tended to reduce with increasing concentrations of smoke product, becoming increasingly significant with increasing rates of Grayson Regen 2000® Seedstarter. Drewes *et al.* (1995) reported that exposure to smoke product (smoke water) at high concentrations was inhibitory to lettuce germination. Observations from our study also suggest that there is a potential to both inhibit and promote the emergence of plant species using higher rates of smoke products. For example, the reduction in emergence of *Austrodanthonia racemosa, Chrysocephalum apiculatum, Leucochrysum albicans* and *Linum marginale*, and the promotion of *Bulbine bulbosa* at the 180 mL m^{-2} application rate (Grayson Regen 2000® Seedstarter)

clearly demonstrate a concentration response to plant-derived smoke. This observation is of relevance to propagators and restorationists, and suggests when using commercial smoke products that care should be taken to establish the most efficacious rates.

The type of smoke formulation used in this study (smoke vermiculite) will be of interest to nursery propagators (Grayson Regen 2000® Germinator) and restorationists (Grayson Regen 2000® Seedstarter) where it can be demonstrated that particular species respond to smoke. Both commercial formulations are relatively simple to handle and apply and their use in both the nursery and the field may enhance seedling emergence. A minor reservation about the use of Regen 2000® Germinator for nursery seedling propagation is that its recommended rate of application does not provide sufficient cover on a seedling tray to produce the other benefits of vermiculite sought by the propagator. For this reason, the volume of vermiculite applied in these experiments was increased with untreated vermiculite of a similar particle size.

Also, under field conditions the potential for plant-derived smoke to stimulate the germination of weed species must be considered. In a study of smoke effects on soil seed bank germination from eucalypt forest in the Hunter Valley of New South Wales, Read *et al.* (2000) found that germination enhancement was not limited to native species. They reported that while a number of native species responded positively to plant derived smoke, four weed species (one annual and three perennials) were also stimulated.

Karrikinolide (the butenolide 3-methyl-2Hfuro[2,3-c]pyran-2-one, KAR_1) has been identified as a germination-active chemical present in smoke (Flematti *et al.* 2004; Chiwocha *et al.* 2009). Identification of this molecule has allowed various research groups to conduct more precise germination experiments using known concentrations of this chemical (Merritt *et al.* 2006). However, the commercialisation of karrikinolide as a product is believed to be some time off (D Merritt pers. com. 2009). In the meantime, testing smoke responses in both indigenous and non-indigenous species using plant derived smoke is important. However, the lack of products that contain known and controllable concentrations of a specific active ingredient will continue to limit the interpretation of results of experiments using generic plant-derived smoke.

Implications for grassland restoration

The three experiments reported in this study investigated the germination and emergence responses of seven grassland species exposed to a selection of temperature and smoke cues. These species were then to be included in a seed mix for a subsequent seeding program. It remains unclear whether investigations of this type allow for accurate predictions of seed behaviour under field conditions. However, the range of responses to germination cues, both within and between species, mean that site preparation and post-sowing planning for the field sowing of complex seed mixes must anticipate protracted and asynchronous emergence. Therefore, the results provided a basis for decisions about seed mix composition, species-specific sowing rates and sowing treatments (e.g. sowing season, whether to apply a smoke treatment).

Our findings add to a growing body of information that is improving the efficiency with which limited seed resources are used. They confirm considerable species-specific variations in responses, and differences/similarities of responses between our results and other studies. To better guide nursery and field seeding decisions, we strongly recommend that nursery propagators and field practitioners should test seed-lot characteristics prior to the initiation of a grassland restoration program.

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References

Baskin C and Baskin J (1998) *Seed Ecology, Biogeography, and Evolution of Dormancy and Germination*. (Academic Press: New York)

Bell DT (1999) Turner Review No.1: the process of germination in Australian species. *Australian Journal of Botany* **47**, 475-517.

Chiwocha S, Dixon KW, Flematti GR, Ghisalberti EL, Merritt D, Nelson DC, Riseborough JA and Smith SM (2009) Karrikins: a new family of plant growth regulators in smoke. *Plant Science* **177**, 252-256.

Clarke PJ, Davison E and Fulloon AL (2000) Germination and dormancy of grassy woodland and forest species: effects of smoke, heat, darkness and cold. *Australian Journal of Botany* **48**, 687-700.

Delpratt CJ (1996) Seed orchard systems for herbaceous indigenous wildflowers. In *IPPS Australian National Conference*, pp. 68-72. (International Plant Propagators Society: Melbourne)

Dixon KW, Roche S and Pate JSP (1995) The promotive effect of smoke derived from burnt native revegetation on seed germination of Western Australian plants. *Oecologia* **101**, 185-192.

Drewes FE, Smith MT and van Staden J (1995) The effect of a plant derived smoke extract on the germination of light-sensitive lettuce seed. *Plant Growth Regulation* **16**, 205-209.

Flematti GR, Ghisalberti EL, Dixon KW and Trengove RD (2004) A compound from smoke that promotes seed germination. *Science* **305**, 977.

Gardiner CA and Midgley SJ (1994) The importance of seed quality in successful revegetation programs. In *National Workshop On Native Seed Biology For Revegetation*. (ACMER: Perth)

Gibson-Roy PG (2000) An investigation of the germination and establishment potential of selected species from the basalt plains grassland community as indigenous seed mixtures. The University of Melbourne, Victoria, Australia.

Gibson-Roy PG (2005) The Grassy Groundcover Research Project. Short Notes. *Ecological Management & Restoration* **6** (1).

Gibson-Roy PG and Delpratt CJ (2006) Seed Resources for Grassland Restoration. *Australian Plant Conservation* **15** (2), June 2006.

Gibson-Roy P, Delpratt CJ and Moore GM (2007a) Restoring the Victorian Western (Basalt) Plains grassland. 1. Laboratory trials of viability and germination, and the implications for direct seeding. *Ecological Management & Restoration* **8**, 114-122.

Gibson-Roy P, Delpratt CJ and Moore GM (2007b) Restoring Western (Basalt) Plains grassland. 2. Field emergence, establishment and recruitment following direct seeding. *Ecological Management & Restoration* **8**, 123-132.

Hitchmough JD (1994) The reconstruction of semi-natural vegetation. In *Urban Landscape Management*, pp. 447-461. (Ed JD Hitchmough) (Inkata: Melbourne)

Huxtable CHA and Whalley RDB (1999) Emergence and survival of three native grass species sown on roadsides on the Northern Tablelands, New South Wales, Australia. *Australian Journal of Botany* **47**, 221-235.

Lloyd MV, Dixon KW and Sivasithamparam K (2000) Comparative effects of different smoke treatments on germination of Australian native plants. *Austral Ecology* **25**, 610-615.

McIntyre S (1990) Germination in eight native species of herbaceous dicot and implications for their use in revegetation. *The Victorian Naturalist* **107**, 154-158.

Merritt DJ, Kristiansen GR, Flematti GR and Ghisalberti EL (2006) Effects of a butenolide present in smoke on light-mediated germination of Australian *Asteraceae* Seed. *Science Research* **16**, 29-35.

Merritt D, Turner SR, Clark S and Dixon KW (2007) Seed dormancy and germination stimulation syndromes for Australian temperate species. *Australian Journal of Botany* **55**, 336-344.

Morgan J (1998) Comparative germination responses of 28 temperate grassland species. *Australian Journal of Botany* **46**, 209-219.

Morgan JW (1999) Have tubestock plantings successfully established populations of rare grassland species into reintroduction sites in western Victoria? *Biological Conservation* **89**, 235-243.

Morgan JW and Lunt ID (1994) Germination characteristics of eight common grassland and woodland forbs. *The Victorian Naturalist* **111**, 10-17.

Morgan JW, Scarlett NH and Rollason TS (1993) Native grassland sites of significance and species rescue on the Western Basalt Plains, Victoria. Australian Nature Conservation Agency, Endangered Species Unit, Canberra.

Mortlock W (2000) Local seed for revegetation: where will all that seed come from? *Ecological Management & Restoration* **1**, 93-101.

Read TR, Bellairs SM, Mulligan DR and Lamb D (2000) Smoke and heat effects on soil seed bank germination for the re-establishment of a native forest community in New South Wales. *Austral Ecology* **25**, 48-57.

Roche S, Dixon KW and Pate JS (1997) Seed aging and smoke: partner cues in the amelioration of seed dormancy in selected Australian native species. *Australian Journal of Botany* **45**, 783-815.

Thomas PB, Morris CE and Auld TD (2007) Response surfaces for the combined effects of heat shock and smoke on germination of 16 species forming soil seed banks in south-eastern Australia. *Austral Ecology* **32**, 605-616.

Tieu A, Dixon KA, Sivasithamparam K, Plummer JA and Sieler IM (1999) Germination of four species of native Western Australian plants using plant-derived smoke. *Australian Journal of Botany* **47**, 207-219.

Willis AJ and Groves RH (1991) Temperature and light effects on the germination of seven native forbs. *Australian Journal of Botany* **39**, 219-228.

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One hundred years ago

THE UTRICULARIAS . — P.von Huetzelburg, in *Flora*, c. (1909) p. 145, gives the results of a study of various species of *Utricularia* and comes to the conclusion that they are truly insectivorous, being able to digest the insects which they catch owing to the secretion of an enzyme and an acid. The hairs which entrap the insects secrete sugar and mucus, but have no digestive action. The bladders are all of similar structure, and the flap closes so tightly, owing to the mucus present, that no insects can possibly get out.

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